THE CLASSIFICATION OF ORGANISMS TERMED *LEPTOTRICHIA (LEPTOTHRIX) BUCCALIS*

I. Review of the Literature and Proposed Separation into *Leptotrichia buccalis* Trevisan, 1879 and *Bacterionema* Gen. Nov., *B. matruchotii* (Mendel, 1919) Comb. Nov.

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Introduction

For a number of years there has been considerable confusion in the nomenclature of certain oral gram-positive filamentous and rod-shaped bacteria which have been included in the genera *Leptothrix* and *Leptotrichia*. This confusion arose from a number of factors, among which are incomplete bacteriological studies, illegitimate nomenclature, varying definitions of the word "filamentous," and ambiguous generic and type species descriptions of the genus *Leptotrichia*. One direct result of the situation is that two entirely different organisms have been termed *Leptotrichia buccalis* in the literature. One of these, essentially rodlike in nature, with the rods generally 10 μ in length, and frequently joined end to end to form long "filaments," was first adequately described by Thjotta *et al.* in 1939 (28) (figure 1). It has since been studied by Bøe and Thjøtta (9), Hamilton and Zahler (19), Davis and Baird-Parker (15) (type 1), Takazoe and Frostell (27*a*), and others. The other, a thick, long, nonsegmented filamentous organism with a "club-head" or bacillus-like body frequently seen attached to one end, was first described by Kligler (22) as *Leptothrix buccalis* (figure 2). Other investigators have since described it as *Leptotrichia buccalis* or simply *Leptotrichia* (6, 7, 20). It has also been studied by Bartels (1), Gilmour and Hunter (18), Davis and Baird-Parker (15) (type 2), and Richardson and Schmidt (25).

The genus *Leptotrichia* was created by Trevisan (29) to accommodate the oral filamentous forms. Nevertheless, these two organisms are obviously too dissimilar to be given the same name. Furthermore, at a time when there is increased frequency of isolation of many oral filamentous organisms, there are diverse opinions of the *Leptotrichia* generic description. It is the purpose of this paper to suggest suitable classifications for the two organisms described above, and in so doing to clarify the status of this genus. Therefore, the inclusion of oral forms in the genus *Leptothrix* will be considered; the literature concerned with the genus *Leptotrichia* and the species *Leptotrichia buccalis* will be reviewed in detail; and the characteristics of both the above organisms will be discussed.

Discussion

The name *Leptothrix buccalis* was originally employed by Robin (26) in 1853 for filamentous forms which he had seen in wet mounts of tooth scrapings and had first described in 1847. Because of their morphological similarity to the aquatic algal organisms placed in the genus *Leptothrix* by Kützing, Robin believed that the filaments from the oral cavity belonged in the same genus, and therefore termed them *Leptothrix buccalis*. His description was as follows:

"Genre *Leptothrix* Kütz. Trichomata tenuissima eramosa, nec concreta. Espéce 14.—*Leptothrix buccalis* Ch.R. Trichomatibus rigidulis, linearibus rectis vel inflexis, non moniliformibus, achromaticus, extremitatibus obtusis, basi in stromate amorpho granuloso adhaerentibus. Long. 0^{mm},020–0^{mm},100, lat. 0^{mm},0005. Hab. In superficie linguae, intervallis dentium, cavo dentium corruptorum, et in succis stomachi et intestini" (26). (Genus *Leptothrix* Kütz. Filaments very thin, not ramified and not coherent. Species 14.—*Leptothrix buccalis* Ch.R. With straight or curved rigid filaments, not moniliform, colorless, with blunt ends, ad-

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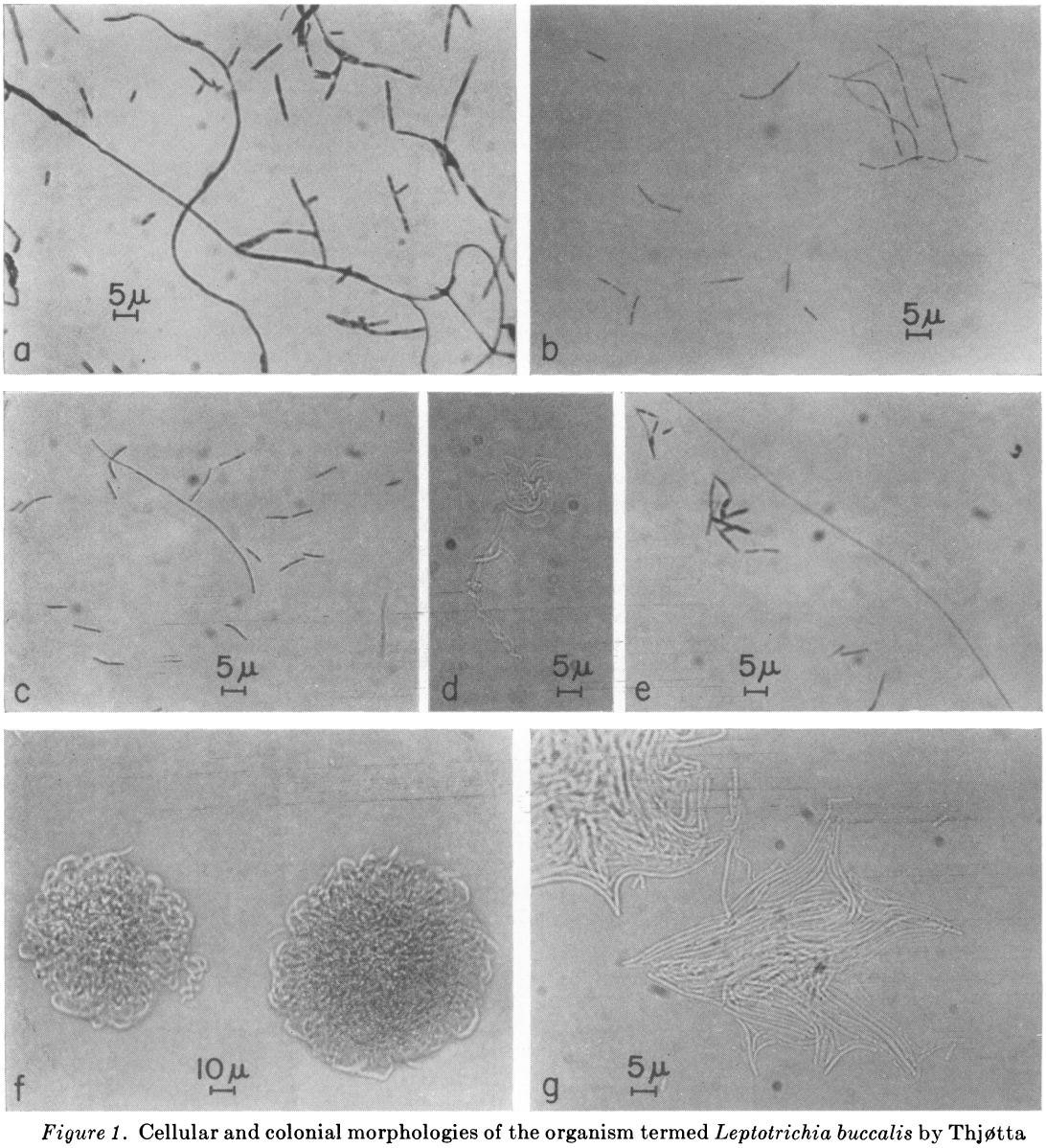

Figure 1. Cellular and colonial morphologies of the organism termed *Leptotrichia buccalis* by Thjøtta *et al.* (28). Isolated from a specimen of calculus. All brain heart infusion agar media supplemented with 0.2 per cent yeast extract. All cultures incubated 37 C.

(*a*) Gram stain of a 12-hr thioglycolate broth culture showing the majority of cells as gram-positive filaments and rods. Some cells which have become almost gram-negative exhibit gram-positive granules.

(*b*) Gram stain from a 48-hr brain heart infusion agar plate grown in 95 per cent N_2 + 5 per cent CO_2, showing trichome formation and gram-negative cells.

(*c*) Gram stain from a 20-hr brain heart infusion agar plate grown in 95 per cent N_2 + 5 per cent CO_2, showing gram-positive rods and short filaments.

(*d*) Unstained microcolony on a 12-hr brain heart infusion agar plate grown in 95 per cent N_2 + 5 per cent CO_2 showing braided appearance of a filament.

(*e*) Gram stain of a 20-hr anaerobic brain heart infusion broth culture showing short gram-positive and gram-negative rods, and one-third of a gram-negative filament.

(*f*) Colonies on a 48-hr brain heart infusion agar plate incubated in 95 per cent N_2 + 5 per cent CO_2.

(*g*) Microcolony on a 16-hr brain heart infusion agar plate incubated under 5 per cent CO_2 + 95 per cent N_2 showing characteristic parallel arrangement of cells.

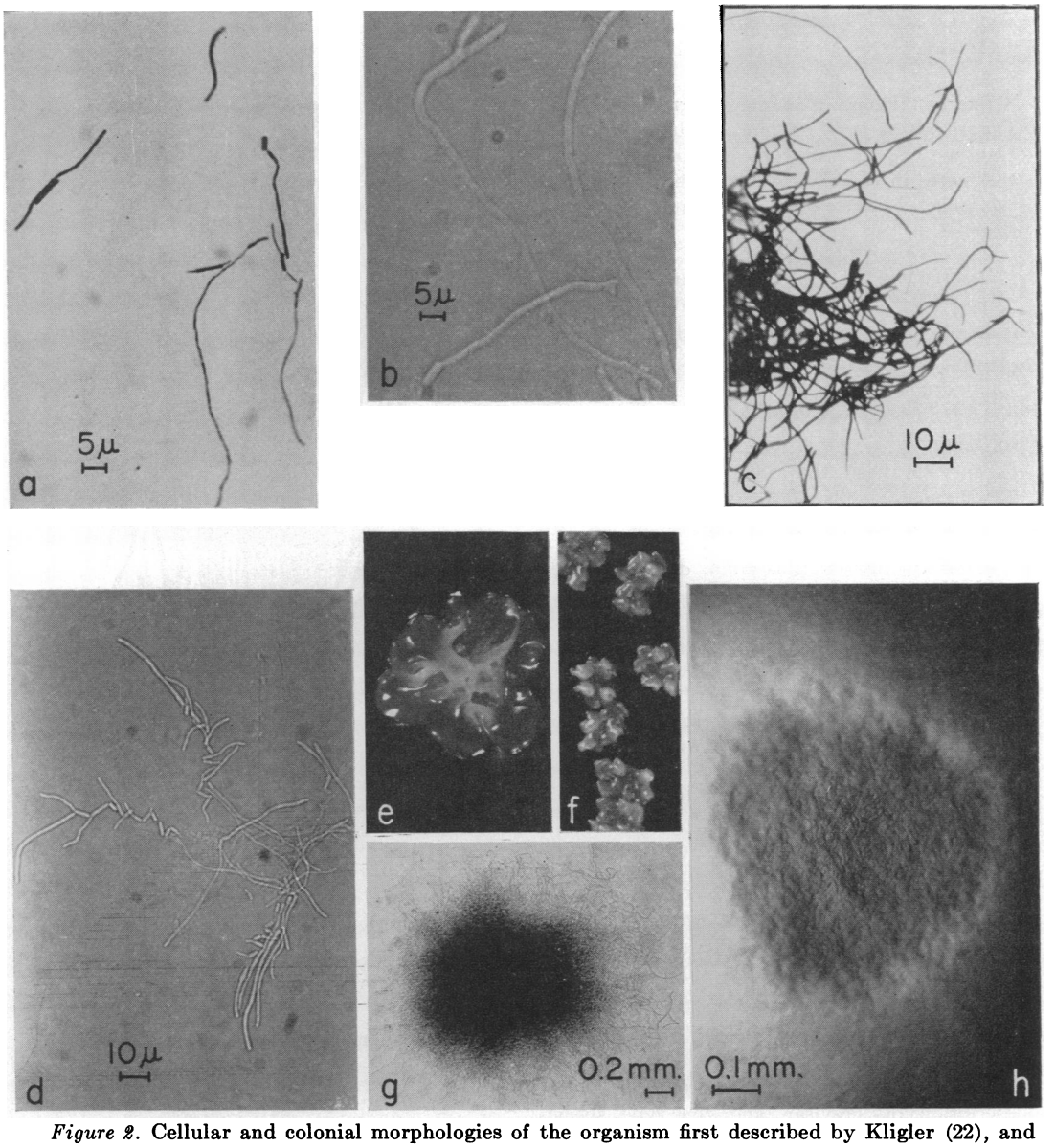

Figure 2. Cellular and colonial morphologies of the organism first described by Kligler (22), and termed *Leptotrichia buccalis* by Bibby and Berry (7) (*Bacterionema matruchotii*). Strains isolated from oral specimens by the authors at the Eastman Dental Dispensary and at the National Institute of Dental Research. All cultures incubated 37C.

(*a*) Gram stain from a 48-hr aerobically incubated plate of brain heart infusion agar supplemented with 0.2 per cent yeast extract. The majority of cells are gram-positive, and the typical variations in cell morphology are shown.

(*b*) Unstained branching cells from a 48-hr aerobically incubated microculture using brain heart infusion agar supplemented with 0.2 per cent yeast extract.

(*c*) Gram stain showing gram-positive cells and dichotomous branching on the edge of a granule from a 48-hr aerobically incubated thioglycolate broth culture.

(*d*) Microcolony on a 24-hr brain heart infusion agar plus 0.2 per cent yeast extract aerobically incubated plate, showing typical cell forms and cell arrangement.

(*e, f*) Typical aerobic streak plate colony morphologies on 4 day incubated plate of brain heart infusion agar supplemented with 0.2 per cent yeast extract (×5).

(*g*) Typical colony morphology in a 48-hr aerobically incubated pour plate of brain heart infusion agar supplemented with 0.2 per cent yeast extract.

(*h*) Colony morphology on a 48-hr aerobically incubated beef extract + starch + blood agar streak plate.

herent at the base in an amorphogranular stroma. Length 0.020 to 0.100 mm, width 0.0005 mm. Habitat. On the surface of the tongue, between the teeth, in the cavity of decayed teeth and in the juices of the stomach and intestine.)[3] (see figure 3.)

In 1879 Trevisan (29) noted that "The generic name *Leptothrix* has been misused so much that it would perhaps be better to discard it completely; in any case it belongs to the Algae. Therefore, in order to change it as little as possible, I shall modify the designation."[3] Accordingly, he proposed the generic name *Leptotrichia* with the type species *L. buccalis* for these oral filamentous forms. The genus *Leptotrichia* was defined by him as follows (29):

"Somatia cylindrica, plus minus distincte articulata, tenuia, elongata, filiformia, recta, laxe fasiculata." (Cylindrical body, more or less distinctly articulated, thin, elongated, filiform, straight, loosely fasiculated.)[3] The type species, *Leptotrichia buccalis*, was described as "*Leptotrichia buccalis* Trevis.; *Leptothrix buccalis* Robin et Lebert. (Zürn, Die pflanzl. Paras. auf und in dem Körp. uns. Haussäugeth., tab. I, fig. 15a)—Sui denti degli uomini e dei cavilli." (On the teeth of man and horses.)

Trevisan's proposal to place the oral filamentous forms in a new genus to separate them from the algae was both valid and useful. However, the descriptions as given were ambiguous. No description was given for the type species, the organism having been neither cultured nor stained, but observed only by Robin in wet mounts from oral scrapings. Additionally, there was a lack of correlation between the generic description of Trevisan and the type species description of Robin. The words *plus minus distincte articulata* in the generic definition suggest that the most common cellular form is a filament-like chain of rods. This conclusion is strengthened by his later description (16). However, the type species, as described and illustrated by Robin (26, 27) indicates a nonsegmented filamentous structure (figure 3).

Trevisan's subsequent taxonomic revisions only led to greater ambiguity. In 1889, he added 13 additional species to the genus *Leptotrichia* and included the genus in the tribe *Leptotrichiee*

[3] Translated by W. H. Everhardy, Translating Unit, Division of Research Services, National Institutes of Health.

Figure 3. Robin's illustration of the organism he observed in wet mounts of tooth scrapings and termed *Leptothrix buccalis* (28; Pl. 1, fig. 1).

(30). However, in the same year De Toni and Trevisan (16) entirely revised the nomenclature of this group. The above 13 species were retained in the genus *Leptotrichia*, whereas the first species to be placed in this genus, *Leptotrichia buccalis* Trevisan (29), was removed to a new genus, *Rasmussenia*, in the tribe *Kurthieae*, and was termed *Rasmussenia buccalis*. The characteristics of the tribe, genus, and species were given as follows (16):

"Tribus III. *Kurthieae* Trevisan.—Sporae (arthrosporae) in filamentis normalibus obvenientes. Filamenta simplicia, basi ab apice superiori ut plurimum distincta, vaginata vel evaginata." "*Rasmussenia* Trev. (Etym. a bacteriologo danico Rasmussen).—Filamenta cylindrica,vagina tenui gelatinosa facile evanescente obducta, simplicia, basi subiculo zoogloeico affixa, articulata, articulis aetate provecta in baculos et

coccos solutis. Multiplicatio baculis primitus vivaciter mobilibus, cito immotis. Arthrosporae transformatione cocci singuli ortae. Obs. *Rasmusseniae* sunt omnes dentium vel oris incolae."

"3. *Rasmussenia buccalis* (Rob. et Leb.) Trev., *Leptothrix buccalis* Robin et Lebert in Robin, Hist. végét. paras. 1847, p. 345, t. I, f. 1–2, Winter, Die Pilze p. 57, Schroet. Pilz. Schles. pag. 171, *Leptotrichia buccalis* Trev. Batt. ital. 1879, p. 14, Gen. p. 10.—Filamentis circ. 1 μ latis, longitudine varia, fragilissimus, saepe in fasiculos coalitis, demum in fragmenta inaequimagna baculiformia vel cocciformia secedentibus; vaginis tenuibus.

"Hab. in dentibus carie exesis.—Salium calcareorum salivae praecipitationem et tartari dentarii formationem inducit. Achroa, jodi ope pallide flavescens; jodi et acidorum actione contemporanea plasma in violaceum tingitur."

"(Tribe III. *Kurthieae* Trevisan.—Spores [arthrospores] occurring normally in filaments. Filaments simple with base well differentiated from apex, with or without sheath. *Rasmussenia* Trev. [Named for the Danish bacteriologist Rasmussen].—Cylindrical filaments, with thin evanescent, wrinkled gelatinous sheath, simple, affixed to the zoogleal base, articulated, with joints broken up into bacilli and cocci at an advanced age. Multiplication by rods at first highly motile but later nonmotile. Arthrospores produced by transformation of the individual cocci.

Observation. *Rasmusseniae* all inhabit the teeth or mouth. *Rasmussenia buccalis* [Rob. and Leb.] Trev., *Leptothrix buccalis* Robin and Lebert in Robin, Hist. végét. paras. 1847, p. 345, vol. I, f. 1–2, Winter, Die Pilze p. 57, Schroet. Pilz. Schles. p. 171, *Leptotrichia buccalis* Trev. Batt. ital. 1879, p. 14, Gen. p. 10.—Filaments about 1 μ wide, of varying length, very fragile, often joined in bundles, and finally contracting into bacilliform or cocciform fragments of unequal size; thin sheaths. Habitat in carious teeth. Causes precipitation of calcareous salts of the saliva and formation of dental tartar. Colorless or colored pale yellow by iodine; plasma stained violet by simultaneous action of iodine and acids.)[3]

In the 1889 De Toni and Trevisan revision (16), the genus *Leptotrichia*, retained for the 13 remaining species, was included in the tribe *Leptotrichieae* Trev. This tribe and genus were described as follows:

"Tribe IV. *Leptotrichieae* Trev.—Sporae nullae aut saltem hucusque numquam detectae. Filamenta simplicia. *Leptotrichia* Trev. (1879) Batter. ital. p. 14, Gen. Pag. 10.—Filamenta cylindrica, vagina tenuissima obducta, articulata, simplicia, basi ab apice superiori distincta, inaequilata, ut plurimum propter pulvinulum mucosum affixa, raro libera, immobilia vel rarissime lente oscillantia. Multiplicatio filamentorum divisione ad unam directionem in articulos mobiles (baculogonidia) secedentium." (Tribe IV. *Leptotrichieae*, Trev.—No spores, or at least not detected thus far. Simple filaments. *Leptotrichia* Trev. (1879) Batter. ital. p. 14, Gen. p. 10.—Cylindrical filaments with very thin wrinkled sheath, articulated, simple, with base separated from upper apex, parallel and usually affixed by means of a small mucous pad, rarely free, immobile or very infrequently slowly oscillating. Multiplication of filaments occurring by division in one direction into mobile joints [baculogonidia].)[3]

Listed among the synonyms of *Leptotrichia* is *Leptothrix* Kütz., 1843.

There are a number of criticisms of the De Toni and Trevisan genus and species, *Rasmussenia buccalis*. The use of the Robin and Lebert 1847 reference was incorrect since Robin first used the name *Leptothrix buccalis* and gave its complete description in 1853 (26). Additionally, although *Leptothrix buccalis* Robin and Lebert was given as a synonym of *Rasmussenia buccalis*, the description of the latter was completely different from that given by Robin for *L. buccalis*. Furthermore, the type species, and only species, given in the original description of the genus *Leptotrichia* was identical to the type species, and only species, given for the subsequently proposed genus *Rasmussenia*. Since the genus *Leptotrichia*, its description and type species were validly published in 1879 (12, 21) and since two generic names cannot be based upon identical type species, the later change to *Rasmussenia* was illegitimate.

The inclusion of oral forms in the genus *Leptotrichia* was generally disregarded and the oral forms, both filamentous and rod shaped, continued to be illegitimately termed *Leptothrix*. This situation, and the meager generic and type species descriptions for *Leptotrichia* resulted in such confusion that the Society of American Bacteriologists appointed a committee to study the problem. In its 1917 report (31) the committee included the genus *Leptotrichia* in the family *Mycobacteriaceae* and defined it as follows:

"Thick, long, straight or curved threads, frequently clubbed at one end and tapering to the other. Gram-positive when young. Threads fragment into short, thick rods. Anaerobic or faculta-

tive. Non-motile. Filaments sometimes granular; non-branching. No aerial hyphae or conidia. Parasites or facultative parasites. The type species is *Leptotrichia buccalis* (Robin) Trevisan."

Despite its attempts to dispel confusion, the committee was unsuccessful. No description of the type species was given other than to indicate that it was the same as the description for the unstained, uncultivated organism termed *Leptothrix buccalis* by Robin (26, 27). Since the committee's generic description did not fit Robin's description, there was no correlation between its description for the genus and the type species.

The following year, Buchanan (12) placed the genus *Leptotrichia* in the family *Actinomycetaceae*, order *Actinomycetales*. His description of the genus is as follows:

"Rod shaped or filamentous cells, nonmotile, unbranched, without aerial hyphae or conidia; parasites or facultative parasites. The type species is *Leptotrichia buccalis* (Robin) Trevisan."

Again, the type species description was the meager one given by Robin (26, 27). Additionally, the wording of the Winslow and Buchanan descriptions is such that they may be regarded as either similar or dissimilar. It is more unfortunate that the type species chosen for both was the uncultivated, unstained organism originally described by Robin in wet mounts from tooth scrapings. Because the description of this organism is so meager it is impossible for anyone to state that the organism he is studying *in vitro* is that described by Robin.

The first edition of *Bergey's manual of determinative bacteriology* (2) agreed with the Winslow Committee in its description of the genus. *Leptotrichia* was placed as the first genus in the family *Actinomycetaceae*, order *Actinomycetales*. Its generic description is almost identical with that of the Winslow report and was given as follows:

"Thick, long, straight or curved filaments, unbranched, frequently clubbed at one end and tapering to the other. Gram-positive when young. Filaments fragment into short, thick rods. Anaerobic or facultative. No aerial hyphae or conidia. Parasites or facultative parasites. The type species is *Leptotrichia buccalis* (Robin) Trevisan. 1. *Leptotrichia buccalis* (Robin) Trevisan. Rods: 1.0 to 1.5 by 1.6 to 2.0 microns, frequently in long chains, often with partition walls. Nonmotile. Gelatin colonies: Circular, grayish-white, translucent. Gelatin stab: liquefaction crateriform. Agar colonies. Agar slant: White, translucent, becoming yellow, dry. Broth: Flocculent and granular sediment. Litmus milk: Unchanged. Potato: Dirty, white, flat, spreading. No gas in dextrose. Indol not formed. Nitrates not reduced. Anaerobic, facultative. Optimum temperature 35° to 37° C. Habitat: Oral Cavity."

The above generic delineation does not include the *plus minus distincte articulata* nor *laxe fasiculata* characteristics of Trevisan's (29) original definition of this genus. Nor does it correlate with its type species description. In fact, this description well fits an organism reported by Kligler (22) in 1915 as:

"A thick, long, straight, or curved thread with a club-head at one extremity and a tapering end at the other. It is generally 0.8–1 μ thick and upwards of 10 μ in length. *Chromology.* It stains readily with anilin dyes in young cultures. In older cultures it has the appearance of a faintly stained sheath enclosing a number of heavily stained granules. Young cultures are Gram-positive. In older cultures the sheath is decolorized while the granules retain the stain. It is not pleomorphic but the threads fragment very early into short, thick rods. Coccoidal forms are not seen. *Biological properties.* They are anaerobic, facultative-aerobic, non-motile, non-branching threads. They grow at 37°C and practically not at all at 20°C."

The type species description in the 1923 edition of Bergey's Manual (2) did not correspond to the generic description, nor did it conform in any way to Robin's description of *Leptothrix buccalis*. It was in fact based upon the description given by Vignal in 1886 of an organism which "was in all probability one of the common spore-formers" (10).

Despite these inconsistencies, the definitions of *Leptotrichia* and *L. buccalis* given in the first edition of Bergey's Manual (2) were perpetuated in several subsequent editions (3–5). In the sixth edition (10), the genus *Leptotrichia* Trevisan was no longer recognized as a valid genus, and in the seventh edition (11), the genus was ignored. However, it should be noted that in 1954, the Judicial Commission (21) had ruled that the genus should be conserved with *L. buccalis* as the type species.

In 1935 and 1939 Bibby (6, 7) expressed

dissatisfaction with the discrepancies between the generic and type species descriptions in the fourth edition of Bergey's Manual (4). Because the Trevisan descriptions were meager and based upon unstained, uncultured microorganisms, he advised acceptance of the generic definition proposed by the Winslow Committee and employed in Bergey's Manual, and the selection of a type species which would correlate with this generic description. For *Leptotrichia buccalis*, the type species, he suggested the Kligler organism, with the additional biochemical characteristics of fifty such strains which he had studied in some detail.

Thjøtta, Hartmann, and Bøe (28) were also dissatisfied with the Bergey descriptions because "even the description of Bergey in his 5th edition of the Manual of Determinative Bacteriology gives characteristics not identical to those found by Kligler, Wherry and Oliver, whose descriptions must be regarded classical for this microbe" (*i.e.*, *L. buccalis*). In this paper, Thjøtta *et al.* reported characteristics of 10 oral isolates which they regarded as "in all essential properties identical to the classical descriptions of the so-called "*Leptothrix*" or *Leptotrichia* (Trevisan)," and proposed that these strains be termed *Leptotrichia buccalis*. Curiously, they failed to recognize the differences between their strains and those of Kligler. On the basis of these studies and Bøe's (8), Bøe and Thjøtta in 1944 (9) redefined the genus *Leptotrichia* to conform with the description of the type species, *L. buccalis* given by Thjøtta *et al.* in 1939.

"The genus *Leptotrichia* consists of straight or slightly curved rods, mostly about 10 microns in length, and 0.7–1.2 microns in diameter. The length may, however, vary to a great extent. One may find rods of 3 microns and in fluid cultures long filaments of up to 200 microns. As a rule, however, two rods are found together, connected and forming a short filament. The rods are smoothly formed with a slight tapering to the ends. There is no club-formation. Often two organisms are found in a position that very much reminds of a *Fusobacterium* in the typical position of this microbe. The *Leptotrichia* is Gram-positive in very young cultures but it looses this character very soon and thus the older cultures will show negative rods. . . .All *Leptotrichia* strains are anaerobic or microaerophilic microbes" (9).

In part this generic description fits that of Trevisan (29) for the genus *Leptotrichia*. Although Trevisan did not specify the existence of rods, he did note the trichome appearance and the loosely fasciculated characteristic of the Thjøtta form (figure 1). However, the Bøe and Thjøtta generic description, like Trevisan's, does not conform to Robin's description of the original *Leptothrix buccalis*.

From the above discussion, it is understandable why the name *Leptotrichia buccalis* has been applied to both the Kligler form and Thjøtta form of organisms. As indicated above, this name was originally proposed by Trevisan in 1879 for an organism previously termed *Leptothrix buccalis* by Robin in 1853. As noted by Buchanan (12, 13), Robin's usage of *Leptothrix* was illegitimate since the organism described by him was unrelated to the *Leptothrix* first described by Kützing. Trevisan's decision to remove the oral filamentous forms into a separate genus, *Leptotrichia*, would have clarified the nomenclature of this group but for the following factors. First, his definition of this genus did not correlate with its type species description as given by Robin. Second, both the generic and particularly the type species descriptions were inadequate. Because of the continued confusion regarding the nomenclature of the oral filamentous organisms, the Winslow Committee redefined the genus in 1917. Their description of this genus was almost identical to that given by Kligler for *Leptothrix buccalis*, a filamentous organism which conforms neither to Trevisan's generic delineation for *Leptotrichia* nor to Robin's description of *Leptothrix buccalis*. Although the Committee chose *Leptotrichia buccalis* (Robin) Trevisan as the type species, no further type species description was given. Buchanan also redefined the genus in 1918, placed it in the family *Actinomycetaceae*, order *Actinomycetales*, and proposed *Leptotrichia buccalis* (Robin) Trevisan as the type species with no additional description. In 1923, Bergey's Manual accepted the Winslow Committee's description of this genus, but unfortunately gave a wrong description for *Leptotrichia buccalis*. They adopted as the type species, *L. buccalis*, an organism entirely different from the generic definition. Bibby (6) pointed out this discrepancy in Bergey's Manual and suggested that the type species description be changed such that Kligler's organism be designated *Leptotrichia buccalis* (7). In 1939, Thjøtta *et al.* noted that in Bergey's fifth edition (5) "the characteristics given by Bergey to this

genus are obviously incorrect at certain points." Failing to recognize the differences in Kligler's isolate and believing that the organisms they had isolated from the mouth conformed to the classical descriptions of *Leptotrichia*, they termed such isolates *Leptotrichia buccalis*, and in 1944 Bøe and Thjøtta redefined the genus to conform with their 1939 description of *L. buccalis*. Subsequently, organisms similar to the Kligler type have been termed *Leptotrichia buccalis* by Howell and Rogosa (20), whereas the Thjøtta type has been termed *Leptotrichia buccalis* by Hamilton and Zahler (19) and Davis and Baird-Parker (15), and *Leptotrichia* by Richardson and Jones (24).

Although the genus *Leptotrichia* and its type species, *L. buccalis*, are currently ignored in Bergey's Manual (11) this genus and its type species are available for nomenclature purposes. The name *Leptotrichia*, its generic definition, and its type species *Leptotrichia buccalis* were validly published by Trevisan in 1879. Furthermore, the name *Leptotrichia* is considered one of the *nomina generum conservanda* (21). Additionally, it has been suggested (17) that "If *Leptotrichia buccalis* is one of the lactic acid rods, the generic name *Lactobacillus* becomes a later homonym. If the species is regarded as belonging to the *Actinomycetales*, the name *Leptotrichia* is certainly entitled to consideration."

This name can, then, legitimately be used, but its current application to both the Thjøtta and the Kligler forms is impossible since these organisms are quite distinct.

a. A comparison of their properties from the data presented by Thjøtta, Hartmann, and Bøe (28) and Hamilton and Zahler (19), and those presented by Kligler (22), Bibby (6), and Bibby and Berry (7) shows these two organisms to be morphologically and biochemically distinct.

b. Such differences have also been catalogued by Davis and Baird-Parker (15) and by Richardson and Schmidt (25).

c. The morphological features of each given in figures 1 and 2 are distinctive.

The Thjøtta type of organism occurs as rods, filaments, and more frequently as short or long trichomes having a tendency to lie parallel in microcolonies. In very young microcolonies, the filaments often have a braided appearance. Branching has not been observed. Although Bøe and Thjøtta (9) define *Leptotrichia* as anaerobic or microaerophilic, some strains, following these initial isolation conditions, grow well on aerobic transfers (19, 28). The Kligler organism has a basically filamentous cell structure, the filament generally being attached to a bacillus-like body. The organism forms a mycelial growth, may exhibit true branching, and has been termed anaerobic to microaerophilic, facultative, microaerophilic to aerobic, and aerobic. As noted by Davis and Baird-Parker (15) other major differences also exist. These two forms therefore cannot be given the same epithet, and in fact, the present authors believe that they are too dissimilar to be included in the same genus.

PROPOSALS

Because of usage, it seems appropriate to name either the Thjøtta or Kligler form *Leptotrichia buccalis*, to describe it adequately as a type species, and to give a correlating generic definition for the genus. This view apparently follows the above suggestion of the Editorial Board (17). Since Trevisan's original generic definition was validly published (29), and has been accepted by the Judicial Commission (21) it has priority over later definitions. However, in Opinion 13 (21) of the Judicial Commission, *Leptotrichia buccalis* (Robin) Trevisan was conserved as the type species of the genus. As noted above, *Leptothrix buccalis* Robin, employed by Trevisan as his type species for *Leptotrichia*, does not correlate with his generic description, although the DeToni and Trevisan description of *Rasmussenia buccalis* (16), a later synonym of Trevisan's 1879 *Leptotrichia buccalis* Trevisan, does so. It must therefore be assumed that the description of *Rasmussenia buccalis* DeToni and Trevisan (16) also applied to *Leptotrichia buccalis* (Robin) Trevisan 1879. This seems to warrant a change in the designation of the type species from *Leptotrichia buccalis* (Robin) Trevisan (21) to *Leptotrichia buccalis* Trevisan.

Therefore, it appears logical to choose that organism most similar to Trevisan's generic definition as *Leptotrichia buccalis* and to amend his generic definition by the addition of further salient characteristics. As noted earlier, Trevisan's generic definition, when translated, is as follows:

Cylindrical body, more or less distinctly articulated, thin, elongated, filiform, straight, loosely fasiculated (29).

The Thjøtta form (figure 1) has all of these characteristics, whereas the Kligler form does not generally occur with the cells more or less dis-

tinctly articulated, nor are the cells loosely fasiculated (figure 2).

For these reasons, we propose that Trevisan's (29) definition of the genus *Leptotrichia* be amended by the addition of the characteristics given by Bøe and Thjøtta (9) with *L. buccalis* Trevisan as described by Thjøtta *et al.* (28), Bøe and Thjøtta (9), and Hamilton and Zahler (19), as the type species. Furthermore, we agree with Hamilton and Zahler (19) that this genus should be included in the family *Lactobacillaceae*, tribe *Lactobacilleae*.

We therefore propose the following definition of the genus and type species:

Leptotrichia Trevisan, 1879. Straight or slightly curved rods with blunt or pointed ends. In young cultures two rods are generally found together forming a short "filament." In older cultures long chains of cells or filaments up to 200 μ in length may be found. Gram-positive in young cultures, gram-negative in older cultures, older cells often containing gram-positive granules. No evidence of club-formation, branching, motility, or spore formation. Anaerobic or microaerophilic on first isolation, some strains subsequently grow aerobically. Catalase negative. Habitat: Oral cavity.

1. *Leptotrichia buccalis* Trevisan, 1879 (*Leptotrichia buccalis* Trevisan, Reale Istituto Lombardo di Scienze e Lettere, Ser. II, *12*, 133, 1879; *Leptothrix innominata* (Miller) in Wherry and Oliver, J. Infectious Diseases, *19*, 299, 1916; *Leptotrichia buccalis* Trevisan in Thjøtta, Hartmann, and Bøe, Avhandl. Utgitt. Norske Videnskaps-Akad, Oslo I. Mat. Naturv. Kl., No. 5, 1939; *Leptotrichia buccalis* in Bøe, Skrifter Norske Videnskaps-Akad. Oslo I. Mat. Naturv. Kl., No. 9, 1941; *Leptotrichia* in Bøe and Thjøtta, Acta Pathol. Microbiol. Scand., *21*, 441, 1944; *Leptotrichia buccalis* Trevisan in Hamilton and Zahler, J. Bacteriol., *73*, 386, 1957; *Leptotrichia* in Richardson and Jones, J. Dental Research, *37*, 697, 1958; *Leptotrichia buccalis* in Davis and Baird-Parker, Brit. Dental J., *106*, 70, 1959). Rods, 5 to 15 μ long and 1 to 1.5 μ wide, often with pointed ends. Two or more rods frequently joined together to form a short filament; long chains of cells or filaments frequently found. Gram-positive when young, gram-negative in older cultures. No branching. Nonmotile. No spores. Ferments glucose, maltose, sucrose, levulose, mannose, and usually salicin and trehalose with the production of acid but no gas. Dulcitol, mannitol, rhamnose, sorbitol, and xylose not fermented. Acid may be produced from galactose, lactose, glycerol, raffinose, and starch. Voges-Proskauer test negative. Gelatin not liquefied. Indole not produced. Nitrate may be reduced to nitrite. Lactic acid (racemic mixture) produced from glucose. Anaerobic or microaerophilic on first isolation. Some strains grow aerobically on later transfers. Catalase negative.

Were the Kligler type of organism termed *Leptotrichia*, the words *plus minus distincte articulata* and *laxe fasiculata* would have to be deleted from Trevisan's (29) generic definition, his description clarified by the addition of that given for the genus in the Winslow Report (31), which was adopted by Bergey's Manual (2–5), and the latter altered to allow for branching and facultative growth.

Because of its mycelial growth, gram-positive filamentous morphology, and ability to branch, the Kligler organism must be placed in the order *Actinomycetales*, family *Actinomycetaceae*. However, as previously indicated (20, 25), it differs extensively from the two existing genera, *Actinomyces* and *Nocardia*.

We therefore propose that a new genus, *Bacterionema*, be created for the organisms described by Kligler (22), Mendel (23), Bulleid (14), Bibby (6), Bibby and Berry (7), Bartels (1), Gilmour and Hunter (18), Howell and Rogosa (20), Davis and Baird-Parker (15) (type 2), and Richardson and Schmidt (25). The type species is *Bacterionema matruchotii*. This genus may be defined as follows:

Genus *Bacterionema* gen. nov. (Gr. fem. noun *bacteria*, a staff or long rod; Gr. noun *nema*, a thread; *Bacterionema*, a thread-shaped long rod.)

Thick, long, straight or curved nonseptate filaments frequently having a bacillus-like body attached to one end. Dichotomous branching is frequently seen in microcolonies on brain heart infusion agar with or without 0.2 per cent yeast extract incubated 24 hr under 5 per cent CO_2 + 95 per cent N_2 at 37 C. Gram-positive. Reproduction by fragmentation. Facultative. Occasional strains anaerobic. Habitat: Oral cavity.

1. *Bacterionema matruchotii* (Mendel, 1919) comb. nov. (*Leptothrix buccalis*, in Kligler, J. Allied Dental Soc., *10*, 282, 1915; *Cladothrix matruchoti* Mendel, Compt. rend. soc. biol., *82*, 583, 1919; *Leptothrix buccalis* in Bulleid, Brit. Dental J., *46*, 289, 1925; *Leptotrichia buccalis* in Bibby and Berry, J. Bacteriol., *38*, 263, 1939;

Leptotrichia buccalis in Howell and Rogosa, J. Bacteriol., *76*, 330, 1958; *Leptotrichia dentium* Davis and Baird-Parker, Brit. Dental J., *106*, 70, 1959). Thick, long, straight or curved, nonseptate filaments, 1 to 2.3 μ in diameter, 20 to 200 μ in length, frequently showing dichotomous branching in microcolonies on brain heart infusion agar with or without 0.2 per cent yeast extract incubated 24 hr under 5 per cent CO_2 + 95 per cent N_2 at 37 C. Rectangular bacillus-like bodies, usually 1.5 to 2.5 μ by 3 to 10 μ, often occur at one end. Gram-positive. Reproduces by fragmentation of the filaments into rectangular bodies. On serial transfer on a casein hydrolyzate + yeast extract + starch + glucose medium, some strains may lose their filamentous nature and become predominantly bacillary, with the cells varying from 0.5 to 1.2 μ by 2 to 13 μ. Clubbed forms may be evident. Ferments glucose, sucrose, maltose, mannose, and levulose with the production of acid but no gas in Durham tubes; may or may not ferment raffinose, salicin, starch, and dextrin; no acid or gas on arabinose, dulcitol, inositol, glycerol, mannitol, rhamnose, sorbitol, trehalose, and xylose; occasional strains may ferment galactose, inulin, and lactose. Nitrate reduced to nitrite. Voges-Proskauer test usually positive; no hydrogen sulfide produced. Gelatin not liquefied. Indole not produced. Aerobic, facultatively anerobic. Catalase positive.

This view on the nomenclature of this microorganism varies widely from that presented by Davis and Baird-Parker (15), who employ the name *Leptotrichia* for a genus which is otherwise unplaced in a taxonomic scheme. The first species of this genus, *Leptotrichia buccalis*, is the organism described by Thjøtta, Hartmann, and Bøe (28) and the second, termed *Leptotrichia dentium*, is similar to that described by Kligler (22). As indicated above, the present authors feel that the two types of organisms are too dissimilar to be included in the same genus.

Representative cultures of each organism including neotype strains have been deposited in the American Type Culture Collection.

Conclusions

1. The name *Leptotrichia buccalis* Trevisan, 1879 should be reserved for the organism described as *Leptotrichia buccalis* by Thjøtta *et al.* (28).

2. *Leptotrichia buccalis* Trevisan, 1879 should be placed in the tribe *Lactobacilleae*, family *Lactobacillaceae*.

3. It is proposed that a new genus, *Bacterionema*, with *Bacterionema matruchotii* (Mendel, 1919) comb. nov. as the type species, be created for the organism first adequately described as *Leptothrix buccalis* by Kligler (22).

4. *Bacterionema matruchotii* should be placed in the family *Actinomycetaceae*.

Addendum

Since this manuscript was accepted for publication Omata and Braunberg (J. Bacteriol., **80**, 737–740, 1960) have suggested that *Fusobacterium fusiforme* (Veillon and Zuber, 1898) Hoffman (11) should be classified as *Leptotrichia buccalis* Trevisan. We agree with this conclusion.

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